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(54) Title: COMPOSITIONS COMPRISING CONJUGATED LINOLEIC ACID (CLA)

(57) Abstract: The present invention relates to new oral compositions comprising CLA in combination with food grade antioxidants and the use of said combination for the manufacture of a dietetic composition or a medicament useful in the treatment of atherosclerosis, overweight and in enhancing the immune response.

COMPOSITIONS COMPRISING CONJUGATED LINOLEIC ACID (CLA).

FIELD OF THE INVENTION

The present invention relates to new compositions comprising conjugated linoleic acid (CLA).

More particularly, the invention relates to new oral compositions comprising CLA in combination with food grade antioxidants.

The present invention further relates to the use of a combination of CLA and food antioxidants for the manufacture of a dietetic composition or a medicament useful in the treatment of atherosclerosis, overweight and in enhancing the immune response.

BACKGROUND OF THE INVENTION

Conjugated linoleic acid (CLA) is a mixture of positional and configurational isomers of octadecadienoic acid, which are naturally occurring substances found in milk and diary products as well as in meats of ruminants.

The term CLA includes the family of positional and configurational isomers of C18:2 fatty acid, more precisely the cis and trans form of 9,11- 10,12- and 11,13-octadecadienoic acids.

Many studies reported that synthetic CLA is an effective agent in inhibiting mammary, colon, forestomach, and skin carcinogenesis in experimental models, due to its modulation of lymphocyte and macrophage activities. Recent clinical and in vivo data disclosed novel biological effects of CLA, e.g. the anti-atherogenic and anti-hyperinsulinemic activities.

After having attracted the attention of the international

scientific community for its therapeutic properties above, CLA is gaining further consumer acceptance as nutritional supplement as it has been shown that a CLA-enriched diet produces a significant improvement in overall health conditions.

The Wisconsin Alumni Res. Inst. (Madison, WI, USA) disclosed several therapeutic methods based on oral administration of CLA, namely for reducing the secretion of apolipoprotein B (WO98/37873); for elevating CD-4 and CD-8 cell levels (EP0831804); for preventing weight loss and anorexia (US5430066); for mitigating allergic responses (WO97/32008 and EP0883681); for enhancing the activity of natural killer lymphocytes (WO98/19675); for reducing body fat (US5554646 and US5855917) whereas WO97/46118, in the name of the same Institute, disclosed a dietetic food comprising CLA.

US5756469 and US5716926 disclose a composition of CLA and pyruvate and/or anti-cortisol compounds for increasing body protein content.

WO99/12538 discloses a method for the inhibition of liver fat accumulation so as to prevent chronic hepatitis and hepatic cirrhosis by using CLA.

WO99/08540 discloses a functional food containing CLA and omega-3 fatty acid.

CLA is also known as a slimming agent, whose oral consumption produces a marked decrease of body fat with increase in the lean body mass. The effects of CLA on body fat/lean ratio seem to be due to inhibition of both proliferation and differentiation of preadipocytes,

as observed by Brodie A.E. et al. in J. Nutr. 129:602-6 (1999).

We have now find out that a long-term oral intake of CLA is associated with an increasing risk of lipoperoxidative burdens.

According to our findings, high body levels of CLA lead to the formation of cytotoxic aldehydes and acyloins, which are the breakdown products of the aldehyde metabolism. Our data thus indicate that the consumption of CLA lead to a significant enhance of lipoperoxidative stress.

Lipid peroxidation is caused by the reaction between oxygenated free radicals (oxyradicals) and polyunsaturated lipids and play a significant role in ageing and in the pathophysiology of a number of human diseases such as atherosclerosis, cancer and heart disease.

The superoxide ion ($O_2^{\circ-}$) is the most common ion among oxyradicals, and it is characterized by a fair reactivity, which in turns allows it to diffuse and propagate into a variety of biological targets. $O_2^{\circ-}$ is mainly generated as by-product of Krebs pathway, as 1-2% of the oxygen consumed by cells undergoes to the reaction: $O_2 + e^- \rightarrow O_2^{\circ-}$, or by phagocytes as defensive tool against infections via NAOP oxidase reaction, as $O_2^{\circ-}$ tends to kill hosted bacteria and virus.

The $O_2^{\circ-}$ may acquire a further electron to form hydrogen peroxide (H_2O_2) and the two chemical entities can combine to generate a further strong free radical, i.e. the hydroxyl radical (OH°), which is about one thousand times more reactive than $O_2^{\circ-}$, thus being indicated as one the most dangerous radical to human health, the

formation of OH° being assisted by iron in both the Haber-Weiss reaction ($H_2O_2 + O_2° \rightarrow OH^- + OH° + O_2$) and in the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow OH^- + OH°+ Fe^{3+}$).

These oxygenated radicals promptly react with the DNA chain, proteins, LDL and hormones. When lipids are affected a chain propagation starts with the lipid radical (L°), which isomerize to conjugated lipid and/or react with oxygen to produce peroxyl radical LOO°, then transformed in an hydroperoxide (LOOH), further producing L°, i.e. the chain propagator, and a cyclic peroxide. The latter isomerizes to a cyclic endoperoxide, finally reacting with oxygen singolet ($O_2°$), to form malondialdheyde and other oxygenated lipid fragments.

The production of free radicals connected with the administration of CLA may lead to enhanced lipid peroxidation, thus contributing to several types of toxic injury.

Our observations have been confirmed by the recent work of Basu S., Smedman A., and Vessby B. in "Conjugated linoleic acid induces lipid peroxidation in humans" FEBS Lett. 468(1):33–36 (2000).

DETAILED DESCRIPTION OF THE INVENTION

It is appreciated that in the present specification the term CLA is intended to include either CLA in the form of free fatty acid or its derivatives, such as its phospholipid, its mono-, di- and tri-glycerides, ethers, esters or salts thereof. All derivatives must be physiologically acceptable, i.e. non-toxic derivatives of CLA. Preferred salts of CLA include the metallic soaps of CLA with alkaline and/or earth-alkaline

ions, such as sodium, potassium, or magnesium ions, and the nitrogen-containing salts, such as ammonia, mono-, di- or tri-ethanolamine.

One of the purpose of the present invention is to avoid the toxic effects of CLA, by providing a new composition comprising CLA in combination with a suitable antioxidant agent counteracting CLA peroxidation.

Therefore., according to one of its aspects, the present invention concerns a dietetic and/or pharmaceutical composition comprising an effective amount of CLA and at least one physiologically acceptable antioxidant agent.

According to a preferred embodiment, the physiologically acceptable antioxidant agent is a food grade antioxidant effectively preventing the production of free radicals which intervene in the cascade leading to cell death by apoptosis or necrosis.

The term "food grade antioxidant" designates a product which can be safely administered to a human being or to an animal other than human.

Suitable food grade antioxidants include but are not limited to those found in fruits, vegetables, nuts, seeds, leaves, flowers and bark, and combinations thereof.

The food grade antioxidants for the purposes of the present invention are preferably selected among three main groups, namely flavonoids, lipophilic antioxidant vitamins, and plant phenols.

The flavonoids are a large group of vegetal substances either structurally or biogenetically correlated, which are especially

contemplated within the scope of the present invention.

Suitable flavonoids for our purposes may be classified in four main groups according to their structural feature and/or their natural occurrence, i.e. bioflavonoids, proanthocyanosids, anthocyanins, and isoflavones, as listed herewithafter numbered from (i) to (iv).

(i) Bioflavonoids : Main flavonoids are generally defined "bioflavonoides", a collective term describing the variety of naturally occurring flavones, flavanonols, flavanones, flavonols and flavones with antilipoperoxidant action against lipoperoxidation.

Citrus peels are the common source of flavanones (e.g. hesperetin, naringenin and related glucosides), whilst flavanonols are widespread in plant kingdom, mainly as quercetin and its glycosides, as the 3-rhamnoside (quercetrin) from Aesculum hippocastanum, or the 3-rutinoside (rutin) from grape, tobacco and eucalyptus.

Grape and grape derivatives, such as pomace and wine, contains also flavanonols (e.g. myricetin), flavones (e.g. luteolin) as well as the flavanols (better included in catechin sub-goup).

(ii) Proanthocyanosides : These are a group of flavonoids comprising flavan-3-ols and flavan-3,4-diols, which are converted into anthocyanidins such as cyanidin, delphinidin and pelargonidin by an acid treatment, therefore called by the above name.

The compounds include higher molecular procyanidin, known as oligomeric proanthocyanidins, which are dimers, trimers, tetramers, or decamers of (+)-catechin and (-)-epicatechin.

Catechins from green tea contains the gallic moiety within the

structure and/or as gallocatechin, gallocatechin gallate, epigallocatechin and epigallocatechin gallate.

The flavandiols, also known as leucocyanines, are further proanthocyanisides occurring in several plants, e.g. dihydroquercitin and dihydrokaempferol.

The proanthocyanidins are commonly extracted and purified from the grape seed, pine bark, cocoa and other vegetal sources rich in flavan-3-ols in the free, glucosylated, esterified, or condensed forms.

The oxygen free radical scavenging abilities of catechins is the highest among the class of plant flavonoids. The activity against biochemically generated superoxide anion and hydroxyl radical shows 10 to 20 times higher inhibition than vitamin C and vitamin E, respectively.

(iii) Anthocyanins :_These compounds are present in the petals of flowers, in the leaves of most plants and in colored fruits and vegetables. These 2-phenylbenzopyrylium (flavylium) positively charged molecules bear hydroxy or methoxy groups (e.g. pelargonidin, cyanidin, delphinidin, petunidin, peonidin, malvidin), which may also be substituted as mono, di- and tri-saccharides, and position 3 may be acylated, e.g. with p-coumaric acid.

The preferred source of anthocyanins is grape (pomace, plum), which also contains resveratrol and its condensed polymers, the viniferins.

Chalcones are the biosynthetic precursors of flavonoids and anthocyanins, the latter being slowly reverted to the

corresponding chalcones when kept in neutral or slighly alcaline aqueous solution.

The anthocyanins are endowed, to different extent, of capillary protectant activity coupled with antioxidant activity even higher than catechins.

(iv) Isoflavones : The flavonoids extracted from soybean meal, also known as phytoestrogen, exhibit a wide range of biological properties, besides having a pronounced antioxidant activity. The efficacy of soybean meal in the lowering of the serum total cholesterol levels is strictly correlated with its content of isoflavones, which play a role in the prevention of certain cancers by inhibiting protein tyrosine kinase and angiogenesis. Soy bean flavour is the main source of isoflavones such as genistein, daidzein, glycitein and related glycones, genistin, daidzin, glycitin.

The antioxidant effects of flavonoids has been demonstrated in many in vitro and in vivo investigations. Their inhibitory effects on lipoperoxidation is highly effective due to the inhibition of active complexes capable to initiate peroxidation, at the same time, these complexes retain their free radical scavenging activities. Therefore, flavonoids are able to suppress free radical processes at three stages: the formation of superoxide iron, the generation of hydroxyl (or cryptohydroxyl) radicals in the Fenton reaction and the formation of peroxyradicals.

Besides a strong action against iron burdens, flavonoids may promote several positive activities, e.g. nitric oxide production by vascular

endothelium; inhibition of the synthesis of thromboxane in platelets and leukotriene in neutrophils, modulation of the synthesis and secretion of lipoproteins in whole animals and human cell lines, blocking of tumour growth and inhibition of carcinogenesis in different experimental models.

Among inhibitory mechanisms to account for the effect of flavonoids there is the inhibition of phospholipase A_2 and cyclo-oxygenase, of phosphodiesterase with increase in cyclic nucleotide concentrations, and of several protein kinases involved in cell signalling. On the other hand, the inhibition of enzymatic functions other than oxidases, e.g. inhibition of lipoxygenase and thus prevention of the formation of leukotrienes, may also participate in the cell and tissue protection of flavonoids.

A further group of food grade antioxidants are the lipophilic antioxidant vitamins. By definition, these are natural fat-like substances, which in turns act as passive shield onto the cell membranes, thus defending polyunsaturated lipids against the attack of the oxygenated free radicals.

Main groups of lipophilic antioxidant vitamin are tocopherols, carotenoids, lipoates and ubiquinones, as listed hereby numbered from (v) to (viii).

(v) Tocopherols : The 8 positional and configurational isomers of tocopherol are a primary antioxidant defence in living system, whose effective protection is due to efficient reaction with lipid oxy-radicals in the membrane bilayer, rather than to interception of initiating oxygen

radicals.

The α-tocopherol (vitamin E) is often associated with the β- and γ-isomers in most plants, main industrial source being wheat and soybean oil.

The vitamin E unbalance leads to changes in the composition microsome membrane phospholipid, oxidation, superoxide dismutase (SOD) activity decrease, accompanied with the disorders of lung and liver tissues functional state, thereby connected with a poor regulation of the mechanism of membrane penetration. Either a plain tocopherol or its esters thereof can be used.

(vi) Carotenoids : These compounds are a family of are well known lipid-soluble antioxidants.

The isomers of carotene are α–, β–, γ–, δ–carotene, also known as provitamins A since they are converted into vitamin A by liver enzymes in the human body following their oral intake. Carotenes are mainly found along with lycopene in tomatos, in carrots and palm oil, as well as in the green leaves of several plants

Further form of carotenoids are the xantophylls, such as zeaxanthin, bixine, crocetin, criptoxanthin, rubixanthin, violaxanthin, fucoxanthin, lycophyll and lutein, thus found either in terrestrial plants or in algae.

The oral administration of carotenoids significantly reduce lipid peroxidation, with a close cooperation with tocopherols in preventing the oxidative stress. Either a plain carotenoid or its esters thereof can be used.

(vii) Lipoates : The α-lipoic acid plays an essential role in mitochondrial

dehydrogenase reactions, displaying a powerful antioxidant activity. Lipoates function as a redox regulator of proteins such as myoglobin, prolactin, thioredoxin and NF-kappa B transcription factor, preventing the deficits in nerve blood flow, oxidative stress, and distal sensory conduction, by quenching superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and singlet oxygen. By interacting with vitamin C and glutathione, alpha-lipoic acid improves turn over of α-tocopherol and protects cell membranes.

(viii) Ubiquinones : These compounds, or coezymes Qn, are a group of benzoquinoic substance with a side-chain composed by repeated units of unsaturated isporenoid units, whose number (n) define the specific member.

Naturally occurring ubiquinones are widespread in animals, plants and microorganisms, ranging from coenzyme Q_6 to coenzyme Q_{10}.

Dietary ubiquinones and alpha-tocopherol lead to an increases in ubiquinone content in liver, exhibiting a concerted antioxidant response at cellular level in defence against lipoperoxidation. The reduced form of coenzyme Q_{10} is capable of suppression of the lipid peroxidation even without the contribution of α-tocopherol.

A further group of food grade antioxidants are plant phenols, comprising a variety of naturally occurring phenolic substances.

Illustrative examples of plant phenols are ethoxyquin, tyrosol, hydroxytyrosol and its esters (e.g. oleuropeine, verbascoside) boldine, peanut hull antioxidants, nordihydroguaiaretic acid (NDGA) and its esters, guaiac gum, erythorbic acid and its salts (e.g. sodium

erythorbate), cardanol, cardol, anacardicacid, oryzanol, propyl gallate and gallic esters, trihydroxy butyrophenol (THBP).

Plant phenols may be obtained along with carotenoids from spices and herbs, including but not limited to rosemary, clove, sage, nutmeg, allspice, cinnamon, ginger, pepper, mace, paprika, olive, rice, cashew nutshell, and citrus oils.

Either the naturally occurring plant phenol or synthetic equivalents thereof can be used.

In a preferred embodiment of the present invention, flavonoids, lipophilic antioxidant vitamins and plant phenols are possibly combined with CLA to provide the best balance between lipophilic and amphiphilic balance as well as primary and secondary anti-oxidative protection.

Further food ingredients may be added to the composition to synergistically enhance the activity of food grade antioxidants, namely compounds which are deemed to improve the activity of the food grade antioxidants, said compounds being hereinafter called "co-antioxidant agents"

Examples of co-antioxidant agents are phospholipids, such as egg and soybean lecithin; ascorbates such as ascorbic acid and ascorbyl palmitate; alfa-hydroxy acids such as D,L-lactic, citric, L-tartaric, malic acids.

Therefore, according to another of its aspects, the invention refers to a dietetic and/or pharmaceutical composition comprising an effective amount of CLA, at least one physiologically

acceptable food grade antioxidant agent and one or more co-antioxidant agent.

The content of CLA to be administered in the composition of the invention ranges from 1 to 20 mg of CLA per kg of body weight, the optimum dose depending on the body weight of the subject, and varies according to the age, the sex and the health conditions of the subject. Such a daily dosage of the composition allows the subject to get an effective beneficial amount of CLA. Although there are no particular restrictions concerning the age of the subject and the duration of treatment, it is commonly suggested not to exceed to dose of 3-4 g of CLA/die.

The composition of the invention preferably contains a ratio CLA/food grade antioxidant agent from about 10:1 to about 1:5 by weight, preferably from about 4:1 to about 1:1 by weight. However, the amount of the food grade antioxidant agent in the composition of the invention depends on the type of the food grade antioxidant agent used. For example, as far as the flavonoids and the plant phenols are concerned, it is recommended not to exceed the daily dose of 2 g, whilst for the lipophilic antioxidant vitamins shall preferably administered at the RDA levels.

In one of its preferred embodiment, the composition of the invention comprises from about 35 to about 70 weight %, more narrowly about 45%, CLA; from about 20 to about 5 weight %, more narrowly about 10%, grape seed proanthocyanosides; from about 5 to

about 10 weight %, more narrowly about 7%, citrus bioflavonoids (expressed as quercetin); from about 1 to about 3 weight %, more narrowly about 2%, gallic acid in gallic esters; from about 1 to about 5 weight %, more narrowly about 3%, vitamin E acetate; from about 0.2 to about 0.8 weight %, more narrowly about 0.3%, mixed carotenoids; from about 0.2 to about 1.5 weight %, more narrowly about 1%, alpha-lipoic acid; and from about 0.2 to about 1 weight %, more narrowly about 0.5% coenzyme Q10.

The composition of the invention is preferably administered by oral route in unit dosage forms, preferably in admixture with customary carriers. Such unit dosage forms comprise soft gelatine capsules, tablets or the like, or in bottles and ampoules in liquid or emulsified form.

An illustrative example of a nutritional composition according to the present invention is a dosage form, such as the soft gel capsule, comprising from about 500 mg to about 1000 mg of CLA and from about 100 to 500 mg of antioxidant agent per unit dose. Said compositions may be preferably administered 1-3 times per day.

The composition of the present invention may also include further biologically active ingredients as well as non-toxic inert carrier or diluent or other auxiliary agents, such as taste modifiers, sweeteners, buffers, etc., in admixture with the above mentioned ingredients.

The composition of the invention may be prepared according to conventional pharmaceutical techniques, for examples as

described in "Remington's Pharmaceutical Sciences Handbook", Mack Pub. Co, USA.

Suitable food grade antioxidants and auxiliary agents are preferably those food ingredients and additives as listed in EEC Directive No. 89/107 issued on 21/12/1988 and further amendments.

According to another of its aspects the present invention relates to the use of the use of a combination of CLA and a food grade antioxidant for the manufacture of a medicament useful in the treatment of atherosclerosis, overweight and in enhancing the immune response.

According to another of its aspects the present invention relates to a method for the treatment of atherosclerosis, overweight and for enhancing the immune response which comprises administering to a subject an effective amount of the composition of the invention.

In addition, the composition may also be in a liquid form, such as oily or emulsioned liquid, to be admixed to food or drinks, baby-food, or in further functional foods, either for human such as such as milk or dairy products and for animals such as fodder, chicken-feed and the like, which comprises CLA and food grade antioxidant agents.

The following examples show in detail how the present invention can be practiced but should not be intended as limiting it.

Preparative Example 1 - Synthesis of CLA by alcaline isomerization of grape seed oil in glycerol

1 kg glycerol, 235 g potassium hydroxide (KOH) and 1000 g of

grape seed oil were added into a 4-neck round bottom flask (5000 ml) equipped with a mechanical stirrer, a thermometer, a reflux condenser, and a nitrogen inlet, the nitrogen being introduced in first run through two oxygen traps.

Nitrogen was bubbled into the reaction mixture for 20 min and the temperature was then raised to 90-100 °C, and kept under mechanical stirring for about 20 minutes to convert the trigliceride in the corresponding potassium salts. The double phase system disappears to form a glyceric soap suspension, then heated at 230 °C under inert atmosphere and stirred for 4 hours.

The reaction mixture was cooled to about 100 °C, and the stirring stopped as the reaction mixture tend to reach very high viscosity during cooling. 2 l of water was then slowly added, and the mixture kept at 95°C for 2 hour. This operation becomes necessary because of the neglegible presence of water and high content of glycerol causing fatty acids to be present as mono- and diglyceride from 5% to 10% by weight of the total lipid content. As partial glyceride esters tend to form W/O emulsion, the water addition and re-heating provides full saponification of the residual esterified fatty acid.

The mixture was transferred into a becker, then cooled to room temperature and 50% w/v sulfuric acid was added to the mixture which was stirred for 1 hour until the pH stabilized at about 3.

The acidulated oil phase formed a lower hydroglyceric layer and an upper fatty acid oil layer containing CLA, which was separated by decantating. Noteworthy, in industrial operation the

separation could be carried out by centrifugation.

The CLA was washed with water and finally it was made anhydrous with sodium sulphate and filtered, then it is stored in a dark bottle at 4 °C until time of use. Total yield is about 770 g of an amber oil, whose GC-analysis is shown in Table 1.

The foregoing synthesis makes the object of a co-pending application.

TABLE 1

Fatty Acid	Grape Seed (Starting material)	CLA from Grape Seed (Final Product)
C14:0	0.11	0.13
C16:0	6.53	6.56
C18:0	3.02	3.23
C20:0	0.19	0.20
total saturated	9.85	10.12
C16:1	0.42	0.48
C18:1	16.42	17.15
C18:1(t)	0.08	0.23
C20:1	0.59	0.60
total monounsaturated	17.51	18.46
C18:2	72.11	1.76
C18:2-conjugated (CLA)	0.21	69.48
C18:3	0.31	0.18
C20:3	0.01	0.00
total polyunsaturated	72.64	71.42

The composition of CLA appears to be a complex mixture, i.e. 9c,11t-

and 8c,10t- octadecadienoic acids at 30,90 %, 11c,13t- 10t,12c- octadecadienoic acids at 32,05 %, 11t,13c- 8c,10c- 9c,11c- octadecadienoic acid at 1,55 %, 10c,12c- 11c,13c- 11t,13t , 9t,11t- 10t,12t- 8t,10t-octadecadienoic acids making the remaining part.

Comparative Examples 1, 2 and Applicative Example 1 – Soft gel capsules

Three different soft gel capsules of 1.35 g were prepared by pharmaceutical procedures using food grade ingredients as shown herewithafter:

Ingredient	Capsule of Comparative Example 1	Capsule of Comparative Example 2	Capsule of Applicative Example 1
CLA-free fatty acid of the Preparative Example 1	-	0.8 g	0.6 g
Soybean fatty acids	0.99 g	0.19 g	-
alpha-Tocopherol	0.01 g	0.01 g	0.1 g
beta-Carotene	-	-	0.05 g
Alpha-lipoic acid	-	-	0.25 g
Bees wax	0.10 g	0.1 g	0.1 g
Gelatin	0.25 g	0.25 g	0.25 g

Therefore, the oral formulations contain no CLA, CLA without antioxidants, and CLA with lipophilic antioxidants, respectively.

Applicative Example 2 – Oxidative stress in plasma by the oral administration of CLA alone and in combination with antioxidants

A group of 9 subjects was divided in 3 groups of 3 individuals

each, and administered once a day with a capsule of Comparative Example 1, a capsule of Comparative Example 2, and a capsule of Applicative Example 1, respectively.

The subjects were analized by by the d-ROMs® kit test (IRAM, Parma, Italy). Briefly, 40 ul of blood sample are taken by finger puncture at day 0, 10, and 20 of the treatment. The analysis is carried out immediately after the sampling, the 40 ul capillary is placed in 3,92 ml of acetate buffer solution at pH 4.8 containing. After dissolving in the aqueous media about 40 ul of N,N-diethyl-para-phenylen-diamine where added, the sample was then centrifuged at 3000 rpm for 3 minutes, than placed in a cuvette and heated at constant temperature for 3 minutes, then the absorbance is measured at 505 nm. The results are shown in Table 2.

TABLE 2

Oxygenated radicals in blood on subjects treated with CLA and CLA+anti-oxidants

Subjects treated with capsules	Mean value at day 0 (*)	Mean value at day 10 (*)	Mean value at day 20 (*)
of the Comparative Example 1	227+/-31	235+/-18	221+/-3
of the Comparative Example 2	216+/-44	295+/-24	342+/-51
of the Applicative Example 1			

	231+/-40	246+/-42	238+/-35

(*) The oxidative stress is expressed as Carratelli Units (Carr.U.), whereas 1 Carr.U. equals approximately the concentration of 0.08 mg % of hydrogen peroxide.

The results showed that the level of lipoperoxidative stress was partially restored to the original values by the combined use of CLA and food-grade lipophilic antioxidants.

Applicative Example 2 – Soft gel capsules

100 g of a gel capsules contain:	
CLA	45
grape seed proanthocyanosides	10
citrus bioflavonoids	7
gallic acid in gallic esters	2
vitamin E acetate	3
mixed carotenoids	0.3
alpha-lipoic acid	1
coenzyme Q10	0.5
Edible unsaturated oil q.b.	to 100

Applicative Example 3 – Beverage

100 g of a beverage contains:	
guar gum	0.02
xanthan gum	0.04
propylene glycol alginate	0.07
mono-diglycerides	4.00
dextrose	15.00

CLA	0.30
orange and carrot juices concentrate	2..80
green tea polyphenols 90%	0.20
anthocyanosides form grape	0.10
carotene and lycopene	0.05
alpha-tocopherol	0.10
nordihydroguaiaretic acid	0.05
acylglycerol	0.10
water	q.b. to 100

Applicative Example 4 – Tablets

100 g of tablets contain:	
calcium carbonate	40.00
magnesium stearate	2.50
CLA	1.00
rutin trihydrate	0.50
hesperidin	0.75
quercetin	0.25
coenzyme Q10	0.10
alpha-lipoic acid	0.05
rice oil	0.30
acesulphame	0.20
sorbitol	4.50
flavour	0.50
starch	q.b. to 100%

It should be understood that the specific forms of the invention herein illustrated and described are intended to be representative only. Changes, including but not limited to those suggested in this specification, may be made in the illustrated embodiments without departing from the clear teachings of the disclosure. Accordingly, reference should be made to the following appended claims in determining the full scope of the invention.

CLAIMS

1. Oral composition comprising as active ingredients Conjugated Linoleic Acid or a derivative thereof (CLA) and at least one food grade antioxidant.
2. Oral composition of the claim 1, characterized in that the ratio of CLA to food grade antioxidant ranges from 10:1 to about 1:5 by weight.
3. Oral composition of the claim 2, characterized in that the ratio of CLA to food grade antioxidant ranges from about 4:1 to about 1:1 by weight.
4. Oral composition according to any one of the preceding claims, characterized in that CLA derivatives comprise one or more cis and trans isomers of the 9,11- 10,12- and 11,13-octadecadienoic acids, its phospholipid and its mono-, di- and tri-glycerides, ethers, esters or salts thereof.
5. Oral composition according to any one of the preceding claims, characterized in that the at least one food grade antioxidant is selected in the group consisting in flavonoids, a lipophilic antioxidant vitamin and plant phenols.
6. Oral composition according to claim 5, characterized in that the substance having a flavonoid structure is selected in the group consisting in bioflavonoids, proanthocyanosids, anthocyanins, and isoflavones and mixtures thereof.
7. Oral composition according to claim 5, characterized in that the lipophilic antioxidant vitamin is selected in the group consisting in

tocopherols, carotenoids, lipoates and ubiquinones and mixtures thereof.

8. Oral composition according to claim 5, characterized in that the plant phenols are selected in the group consisting in ethoxyquin, tyrosol, hydroxytyrosol and its esters (e.g. oleuropeine, verbascoside) boldine, peanut hull antioxidants, nordihydroguaiaretic acid (NDGA) and its esters, guaiac gum, erythorbic acid and its salts (e.g. sodium erythorbate), cardanol, cardol, anacardicacid, oryzanol, propyl gallate and gallic esters, trihydroxy butyrophenol (THBP) and mixtures thereof.

9. Oral composition according to claim 1 to 8 in an appropriate liquid forms to be admixed to food or drinks, baby-food, in further functional foods or in food for animals.

10. Oral composition according to any one of the preceding claims, in unit dosage forms.

11. Oral composition according to claim 10 where said unit dosage form comprises from about 500 mg to about 1000 mg of CLA.

12. Use of a combination of CLA and a food grade antioxidant for the manufacture of dietetic composition or a medicament useful in the treatment of atherosclerosis, overweight and in enhancing the immune response.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 00/01277

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/30 A61K31/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 69272 A (UNILEVER PLC ;LEVER HINDUSTAN LTD (IN); UNILEVER NV (NL)) 23 November 2000 (2000-11-23) claims 1,3,4,6,9,11-13 page 1, line 3 - line 13 page 4, line 32 -page 5, line 4 ---	1,4-7,9, 10,12
P,X	WO 99 55326 A (VIT IMMUNE L C) 4 November 1999 (1999-11-04) example 4 --- -/--	1-3,5,6, 9-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 January 2001	17/01/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Heezius, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 12538 A (IMADA TAKUMA ;MASAKI KYOSUKE (JP); NODA TSUNEYUKI (JP); SHIMIZU SE) 18 March 1999 (1999-03-18) cited in the application & EP 1 010 424 A (NODA TSENEYUKI ET AL.) 21 June 2000 (2000-06-21) claims 1-7 example 3; tables 3,4 page 4, line 3 - line 10	1-5,7, 9-12
X	DE 197 18 245 C (HENKEL KGAA) 30 July 1998 (1998-07-30) claims 1-3,5,7,8 column 5, line 7 - line 15 column 5, line 19 - line 30 examples 1,2	1,4-7,9, 10
X	WO 97 18320 A (LODERS CROKLAAN BV ;CAIN FREDERICK WILLIAM (NL); MOORE STEPHEN RAY) 22 May 1997 (1997-05-22) claims 6,8,10,13-15 page 6, line 29 - line 36	1,5, 7-10,12
X	SHANTHA N C ET AL: "Conjugated linoleic acid concentrations in processed cheese containing hydrogen donors, iron and dairy-based additives." FOOD CHEMISTRY., vol. 47, no. 3, pages 257-261, XP002035277 ELSEVIER SCIENCE PUBLISHERS LTD., GB ISSN: 0308-8146 page 257, right-hand column, paragraph 2; table 1	1,4,5,8, 9,12

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INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter. nal Application No
PCT/IB 00/01277

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